Androgens, oestrogens and endometrium: a fine balance between perfection and pathology

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Androgens, oestrogens and endometrium – a fine balance between perfection and pathology

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Androgens, oestrogens and endometrium

Abstract

The endometrium is a complex multicellular tissue that is exquisitely sensitive to the actions of sex steroids synthesised in the ovary (endocrine system). Recent studies have highlighted a previously under-appreciated role for local (intracrine) metabolism in fine-tuning tissue function in both health and disease. In this review we have focused on the impact of oestrogens and androgens on endometrial function summarising data from studies on normal endometrial physiology and disorders including infertility, endometriosis and cancer.

We consider the evidence that expression of enzymes including aromatase, sulphatase and AKR1C3 by endometrial cells plays an important role in tissue function, and malfunction, and discus results from studies using drugs targeting intracrine pathways to treat endometrial disorders. We summarise studies exploring the spatial and temporal expression of oestrogen receptors (ERalpha, ERbeta and GPER) and their role in mediating the impact of endogenous and synthetic ligands on cross-talk between vascular, immune, epithelial and stromal cells. There is a single androgen receptor and androgens play a key role in stromal-epithelial cross talk, scar-free healing of endometrium during menstruation and regulation of cell proliferation. The development of new receptor-selective drugs (SERMs, SARMs, SARDs) has reinvigorated interest in targeting receptor subtypes in treatment of disorders including endometriosis and endometrial cancer and some show promise as novel therapies.

In summary, understanding the mechanisms regulated by sex steroids provides the platform for improved personalised treatment of endometrial disorders as well as novel insights into the impact of steroids on processes such as tissue repair and regeneration.

Androgens, oestrogens and endometrium

Introduction

In women, the endometrium is divided into an inner/luminal functional layer ('functionalis') and a basal layer ('basalis'). On its inner (luminal) aspect, columnar epithelial cells form a boundary between the fluid-filled uterine lumen and endometrial tissue containing glands, a well-developed vasculature, stromal mesenchyme (fibroblasts, perivascular cells) and a diverse population of immune cells. Between menarche and menopause the endometrium responds to fluctuating levels of blood borne ovarian sex-steroid hormones (primarily 17ß-oestradiol (E2) and progesterone (P)), with cyclical proliferation and differentiation ready to support a prospective pregnancy. In a non-pregnant cycle the functional layer is shed during menstruation but within a few days, the luminal surface is healed and tissue integrity restored ready to resume the next cycle (Garry, et al. 2009)

Whilst sex steroid hormones are essential for the maintenance of normal uterine function and fertility, they may also contribute to the development of hormone-dependent endometrial disorders that affect millions of women (Table 1). In this review we have focused on the impact of oestrogens and androgens on the function and malfunction of the endometrium, considering evidence for expression of receptors that can mediate their function as well as enzymes that modulate local bioavailability of steroids. The emergence of new classes of drugs that target receptors or enzymes and offer some potential as novel treatments for endometrial disorders is summarised.

Oestrogen and androgen receptors and their expression in endometrial tissues

Overview of changes in tissue function during the menstrual cycle

Based on evaluation of 8000 endometrial biopsies, Noyes et al. (Noyes, et al. 1975) published a classification of the different stages of the menstrual cycle which is still considered the gold standard for histological staging. Although cycle length can vary between individuals, staging is typically based on an average menstrual cycle of 28 days: menstruation (day 1), proliferative phase (day 4 to 14) and secretory phase (days 16 to 28). Histologically, the functional layer thickens from about 2 mm recorded immediately after the menstrual phase, to 14 mm prior to ovulation on day 14, (Hess et al., 2006). Following ovulation and formation of the corpus luteum (CL) there is a rapid rise in circulating concentrations of P, which stimulates functional transformation of the stromal fibroblasts (decidualisation) resulting in shape change and reprogramming of gene expression leading to secretion of factors that regulate immune cell recruitment and receptivity (see comprehensive review by (Gellersen, et al. 2007). In the absence of a healthy blastocyst, the regression of the CL results in a rapid decrease in the circulating concentrations of ovarian-derived steroid hormones (progesterone withdrawal), and triggers a cascade of changes in endometrial tissue that results in tissue breakdown, piecemeal shedding and synchronous healing during menstruation (Garry et al. 2009).

Structural and functional features of oestrogen and androgen receptors: genomic and non-genomic signalling

Changes in expression of oestrogen and androgen dependent genes are orchestrated by interaction of their receptors with DNA binding domains within gene promoters/enhancers as well as non-genomic signalling pathways initiated at the membrane. Steroid receptors contain three key structure-function domains: a variable amino-terminal domain, a highly conserved DNA-binding domain (DBD), and a less conserved carboxyl-terminal ligand binding domain (LBD). Differences

in the sequence of amino acids located within a C-terminal ligand binding pocket play a critical role in ligand selectivity (Nadal, et al. 2017; Shiau, et al. 1998). A linker region situated between the DBD and the LBD functions as a flexible hinge with a nuclear localization signal: the proteins also contain multiple sites for phosphorylation (Lannigan 2003). There are two oestrogen receptors (alpha and beta) encoded by separate genes, *ESR1* and *ESR2*, respectively: the full length wild-type proteins they encode (hERα and hERß1 respectively) bind a range of oestrogenic ligands with high affinity and specificity. Notably analysis of natural ligand reported that whilst 17ß-oestradiol (E2) bound both receptors with high and equal affinity, oestrone (E1) had higher affinity for wild type ERß(1) (Zhu, et al. 2006). Multiple splice isoforms of both genes have been identified (reviewed in (Gibson and Saunders 2012)). ER46 was the first splice variant of human *ESR1* described (initially designated hERα-46; (Flouriot, et al. 2000). *ESR2* splice variants including ERß2/bcx and ERß5 are co-expressed in multiple reproductive tissues and reproductive cancers (Collins, et al. 2009; Critchley, et al. 2002; Saunders, et al. 2002; Shaaban, et al. 2008). In addition to *ESR1* and *ESR2* a family of closely related genes have been identified as encoding 'estrogen receptor related' proteins (*ESRR1, ESRR2, ESRR3*) which do not bind directly to E1 or E2 as they lack a proper binding pocket at their C-terminus but which may be activated by cofactors or other lipids (reviewed in (Gibson and Saunders 2012; Horard and Vanacker 2003)).

There is a single androgen receptor gene (*AR*) located on the X chromosome. Elegant studies, including those using surface plasmon resonance, have revealed that the long AR N-terminal domain (NTD) is structurally important for receptor-dependent gene expression (Lavery and McEwan 2008) and is a promising drug target (Ponnusamy, et al. 2019). Several splice variant isoforms of AR have been identified with particular attention paid to their role in ligand-independent gene activation in advanced prostate cancers (Dehm and Tindall 2011). Expression of AR variants including AR-V7 (exons 1/2/3/CE3), has also been reported in primary breast cancers and breast cancer cell lines (Hickey, et al. 2015) but a literature search did not identify any data related to their expression in endometrium or endometrial disorders.

There have been extensive studies on the functional consequences of steroid ligand binding to ERs and AR that have been well reviewed elsewhere (Gronemeyer, et al. 2004; McKenna, et al. 1999). Briefly, ligand binding induces a conformational change in the ligand binding domain, dimerization and recruitment of co-regulators that play a critical role in regulating the hormonal response. Ligand-activated receptors can bind directly to DNA sequences within regulatory regions of genes with sequences that are recognised by oestrogen (ERE – oestrogen response elements) or androgen (ARE – androgen response elements) receptors having been described (Brodie and McEwan 2005; Carroll, et al. 2006). Binding studies have also identified a number of so called 'pioneer' factors such as FOXA1 and GATA2 that can enhance direct binding of ER or AR to DNA (Carroll, et al. 2005; He, et al. 2014). ERs also regulate gene expression through protein-protein interactions with other transcription factors already bound on DNA ('tethering') - examples of tethering mechanisms include binding to the transcription factor Sp1 which has been implicated in regulation of the progesterone receptor gene (Petz, et al. 2004) and ERß-dependent induction of gene expression in human endometrial endothelial cells (Greaves, et al. 2013).

Oestrogens and androgens can also induce changes in cell function following binding to ERs or ARs localised in the cell membrane. These 'non-genomic' signalling cascades can be initiated

through the membrane localization of the classical receptors following palmitoylation and interaction with scaffolding proteins or by hormone-responsive G protein-coupled transmembrane receptors (GPCRs) (Hammes and Levin 2007). One of the most extensively investigated GPCRs is GPER (originally named GPR 30, also known as GPER1) which was cloned from breast cancer cells in 1997 and binds oestrogens with nanomolar affinity (Carmeci, et al. 1997). Information on GPCRs that bind to androgens is less comprehensive but several candidates including GPRC6A have been identified in cancer cells (Ye, et al. 2019).

A recent review provided a useful summary of the wide range of different non-genomic signaling pathways and how the different genomic and non-genomic pathways may interact (Wilkenfeld, et al. 2018).

Expression and functional impact of oestrogen receptors during the menstrual cycle

We, and others, have used highly specific antibodies to explore temporal and cell specific patterns of expression of ERα, ERß, ERRs and AR in endometrium during the normal cycle (Critchley and Saunders 2009; Young 2013). We have documented cell specific and temporal immunoexpression of full length ERα (ER66) in both normal endometrium and in endometrial pathologies including cancer (Critchley et al 2002; Collins et al 2008). In full-thickness sections of endometrium (Figure 1) immunoexpression of ERα is intense in the epithelial glands and in the stroma of both the functional and basal layers: endothelial cells lining the blood vessels appear immuno-negative (Critchley, et al. 2001). Expression is down-regulated in the functional layer during the secretory phase in response to the rising levels of progesterone (Figure 1. (Lessey, et al. 1988; Young 2013). We have recently explored expression of ER46 in the endometrium using a combination of immunohistochemistry and Western blotting (Gibson, et al. 2020). Notably the variant protein was co-localised with ER66 in cell nuclei during the proliferative phase with striking expression in a population of uterine natural killer cells (uNK) implicated in vascular remodelling (Gibson, et al. 2015; Quenby, et al. 2009).

Studies in mice suggest a complex role for ERα in epithelial and stromal compartments of the endometrium. For example, the role of epithelial ERα was studied using a conditional knockout mouse which were ovariectomised and then treated with a single intraperitoneal injection of 0.25 µg 17β-estradiol (E2) in 100 µl sesame oil. Analysis of samples recovered 2, 24 or 72h after E2 injection revealed that epithelial ERα was dispensable for the proliferative response observed 2h but essential for responses at 24 and 72h (Winuthayanon, et al. 2014). Similar studies also revealed a critical role for ERα in paracrine regulation of stromal decidualization in this species (Pawar, et al. 2015). The pattern of expression of ERß is distinct from that of ERα with highest concentrations of mRNA encoding full length ERß1 in the secretory phase and immunoexpression in epithelial, stromal, endothelial cells and immune cells (Critchley et al. 2001): ERß1 is not downregulated in the functional layer during the secretory phase (Bombail, et al. 2008). Studies in mice with *Esr2* knockout have suggested a less striking phenotype than in the *Esr1* knockout although a re-evaluation of the evidence by Hapangama et al (Hapangama, et al. 2015) concluded that sustained E2 stimulation of endometrial epithelial cells via ERß might induce apoptosis. There has been some disagreement about the cyclical expression (or otherwise) of ERß in endometrial endothelial cells ((Critchley et al. 2001; Lecce, et al. 2001). A our own study using endothelial cells from different vascular beds demonstrated those originally isolated from endometrium or myometrium were

ERß+/ERα- and revealed cell-specific impacts of an ERß-selective agonist on gene expression (Greaves et al. 2013). In contrast studies using isolated human uNK cells suggest their response to oestrogens may be complex involving rapid membrane-initiated signaling via ER46 (Gibson et al. 2020) and/or binding to ERß (Gibson et al. 2015). Treatment of isolated uNK cells with either oestrone (E1) or E2 promotes cell migration and secretion of chemokine (C-C motif) ligand 2 (CCL2) (Gibson et al. 2015). These studies highlight the importance of endogenous oestrogens in the dynamic interplay between different endometrial cell types that play a critical role in preparation for pregnancy.

Expression of proteins encoded by human *ESR2* splice variant mRNAs (ERß2, ERß5) has been detected in human endometrial cells (Collins, et al. 2019; Collins et al. 2009; Critchley et al. 2002). Notably these variants may also be present in primates (Sierens, et al. 2004) but are not expressed in rodents. In vitro studies have demonstrated the variants can have a functional impact on endometrial cell function by forming heterodimers with full-length isoforms (Collins et al. 2019). Expression of ERRs has also been detected in human endometrium with cell-based studies, highlighting the potential for them to alter cell metabolism or ERα-dependent cell functions (Bombail, et al. 2010a; Bombail, et al. 2010b)

Plante et al (Plante, et al. 2012) examined expression of GPER in endometrium using RT-qPCR and immunohistochemistry reporting maximal expression in the proliferative phase. An earlier study by Kolkova et al (Kolkova, et al. 2010) claimed protein expression was less variable than the mRNA and immuno-staining was more intense in the epithelial cells than stroma throughout the cycle. GPER may be involved in neoplastic transformation of endometrium (Jacenik, et al. 2016) or in promotion of HIF1α-induced expression of MMPs in endometrial stromal cells in women with endometriosis (Zhang, et al. 2017). A number of GPER knockout mice have been generated using different targeting strategies: females are fertile with no obvious reproductive defects although impacts on obesity and vasculature have been claimed (Prossnitz and Hathaway 2015).

Expression and functional impact of androgen receptors during the menstrual cycle

Immunostaining for AR in full thickness endometrial tissue sections (Figure 1, (Marshall, et al. 2011)) detected intense staining in stromal fibroblasts which exhibited cyclical variation in the functional layer but remains unchanged within the basal compartment across the cycle. How this difference in expression within closely adjacent cells is regulated remains unknown. Epithelial cells in the functional layer upregulate expression of AR in response to falling levels of progesterone in a normal cycle or following administration of anti-progestins and this is associated with reduced proliferation (Marshall et al. 2011; Narvekar, et al. 2004). We have identified androgen-regulated genes in primary human endometrial stromal cells several of which (e.g. *CITED2, HIF1a, CD4*4) are implicated in networks that protect cells against stress and apoptosis (Marshall et al. 2011). These data coupled with the observation that AR expression remains unchanged in the stromal cells of basal compartment at time of menses (Garry et al. 2009) prompted us to investigate whether androgens might also play a role in regulating endometrial breakdown and repair using a mouse model that recapitulates key features of menstruation in women (Cousins, et al. 2016a; Cousins, et al. 2016b; Cousins, et al. 2014). In this model, administration of a single injection of DHT at the time of progesterone withdrawal to induce menstruation had a striking impact on both tissue breakdown and restoration of tissue homeostasis. Although our understanding of the role of

androgens in endometrial tissue function is still incomplete, we identified changes in expression of matrix metalloproteinases (MMP3, 9) which are implicated in breakdown of human endometrium (Cousins et al. 2016a).

Expression of enzymes implicated in biosynthesis and metabolism of oestrogens and androgens in endometrial tissue

In recent years there has been a rapid increase in evidence to support a role for local tissue ('intracrine') regulation of endometrial steroids (Gibson, et al. 2013; Gibson, et al. 2018a; Gibson, et al. 2016a). Key findings have included direct measurement of steroids in endometrial tissue homogenates recovered during the menstrual cycle: notably Huhtinen and colleagues reported they did not parallel those in blood (Huhtinen, et al. 2014; Huhtinen, et al. 2012). In women (but not in mice) the adrenals are an important source of sulphated steroids that circulate at high concentrations in the blood but are unable to bind directly to the steroid receptors. A brief summary of enzymes detected in endometrial tissue and their substrates is provided in Figure 2 with a few complementary references discussed below. Readers interested in the topic of intracrine steroids are recommended to read the comprehensive review by Konings et al which includes a systematic search for papers reporting expression of steroidogenic enzymes in pre- and postmenopausal endometrium (Konings, et al. 2018).

Briefly, a strong case has been made that the 'inactive' adrenal steroid dehydroepiandrosterone (DHEA) is an important precursor of bioactive androgens in women (Labrie, et al. 2005), a proposal which has been supported by detection of all the enzymes that regulate conversion of DHEA via intermediates to T, DHT or oestrogens (Gibson et al. 2013; Gibson et al. 2016a; Gibson, et al. 2018b). Catalano and colleagues reported increased expression of *AKR1C3* in the early secretory phase (Catalano, et al. 2011), consistent with results obtained using an in vitro model of stromal decidualisation (Gibson et al. 2016a). Inter-conversion of active/inactive oestrogens and androgens is mediated via 17β-hydroxysteroid dehydrogenase isozymes, of which several isoforms are expressed in endometrium. For example, 17βHSD type 1 is responsible for production of T and E2, from A4 and E1, respectively, whereas 17βHSD2 catalyses the opposite reaction. *HSD17B2*, is expressed in glandular epithelial cells is markedly increased in the secretory phase (Maentausta, et al. 1991) reported overexpression of 17βHSD2 is a feature of endometrium in women with disorders such as endometriosis, adenomyosis, and/or leiomyomas (fibroids) rather than those who are disease-free (Kitawaki, et al. 2000).

Expression of steroid sulphatase (STS) in endometrial tissue can catalyse conversion of DHEAS to DHEA (Figure 2) but can also increase the concentration of E1 by removal of sulphate moieties from E1S. Using an in vitro model of decidualisation we have confirmed expression of both STS and aromatase (*CYP19A1*) in endometrial stromal cells with evidence that both enzymes contribute to production of oestrogens during decidualisation (Gibson, et al. 2018a; Gibson et al. 2013).

Endometrial Disorders: altered expression of enzymes and receptors implicated in disease aetiology

Implantation Failure and Recurrent Miscarriage

Timely and efficient decidualization of endometrial stromal cells in response to ovarian-derived progesterone is essential for the generation of an endometrial microenvironment that can support and nurture the implanting blastocyst. Disruption of decidualization is implicated in implantation failure and miscarriage. Studies in mice using aromatase inhibitors (AI) demonstrated local intra-uterine production of E2 is critical for establishment of pregnancy (Das, et al. 2009). In women, E2 is produced during decidualisation of endometrial stomal cells which regulates uNK cell migration (Gibson et al. 2020; Gibson et al. 2015). Given the evidence that disturbances in the numbers/location of uNK cells can predispose women to experiencing a miscarriage (Lash, et al. 2016) these data are consistent with a role for E2 in regulating the endometrial microenvironment during the establishment of pregnancy.

We have demonstrated that during in vitro decidualisation of primary human endometrial stromal cells there is a significant increase in the expression of AKR1C3, the enzyme responsible for the conversion of androstenedione to testosterone, which is also accompanied by increased secretion of testosterone into the culture medium (Gibson et al. 2016a). In addition, blocking AR action using flutamide during in vitro decidualisation revealed a role for AR-mediated gene expression of osteopontin, a protein implicated in receptivity (Gibson et al. 2016a). Further studies using primary human endometrial stromal cells from women of advanced reproductive age suggested that the age-related decline in adrenal steroids may have an impact on the ability of the endometrium to support a pregnancy and that increased availability of adrenal precursors enhanced androgen production and secretion of decidualisation markers (Gibson et al. 2018b). Intravaginal supplementation with DHEA has shown promising results in alleviating postmenopausal vaginal dryness and atrophy in clinical trials without any adverse effects (Labrie 2018) but delivery into the endometrium of premenopausal women has not been tested. Other studies have reported a positive impact of DHT on stromal cell decidualisation and resistance to oxidative stress (using hydrogen peroxide) (Kajihara, et al. 2012) expanding our understanding of the potentially beneficial role of androgens as direct modulators of endometrial function as well as precursors of oestrogen biosynthesis (see review by (Gibson, et al. 2016b).

Failure to downregulate ERα during the secretory phase (see Figure 1 and discussion above) has been reported in women with defects in uterine receptivity (Lessey, et al. 2006). A complementary study using samples from women with unexplained infertility also showed that in these patients elevated expression of ERα in the mid-luteal phase was associated with reduced expression of glycodelin-A, low levels of which have been implicated in recurrent implantation failure (Dorostghoal, et al. 2018). There is no information about dysregulation of ERß in implantation failure. Fertility problems in women with polycystic ovaries and excess androgens might relate to overstimulation of AR signalling pathways but currently the evidence is quite limited (Schulte, et al. 2015).

Endometrial cancer

The majority of endometrial cancers (EC) present with abnormal endometrial bleeding in postmenopausal women: rates are rising particularly in younger women, with obesity considered a significant contributing factor (Table 1. Reviewed by (Sanderson, et al. 2017). EC are historically classified as type 1 or type 2; type 1 is the most commonly diagnosed form (about 80% of the cases), is considered oestrogen-dependent and characterised by hyperplastic proliferation of the

endometrial glands. A large number of studies have investigated the source and impact of oestrogens in endometrial cancer with landmark papers including those by Sasano and collaborators who reported evidence of increased immunoexpression of aromatase, STS and 17βHSD enzymes in both endometrial hyperplasia and EC (Sasano, et al. 1996; Utsunomiya, et al. 2004; Utsunomiya, et al. 2001). A recent comprehensive systematic review considered the evidence that intracrine metabolism contributes to EC (Cornel, et al. 2019). The authors highlighted the importance of sulphatase and aromatase enzymes in the generation of E1 and E2 within endometrial cancer tissue in promoting a pro-oestrogenic environment favouring proliferation of epithelial cells (Cornel et al. 2019). The authors sounded a note of caution by highlighting the variability between individuals and methodologies which may explain some variations in drug responses (discussed below).

The best evidence for an impact of androgens on EC risk has come from studies in women with polycystic ovarian disease where the risk of type 1 cancers is higher in women with symptoms of androgen excess such as hirsutism and irregular periods (Fearnley, et al. 2010). Tanaka et al reported DHT was elevated in endometrioid endometrial adenocarcinoma tissues compared with that in normal endometrial tissues (8.0-fold) in a group of 41 patients (Tanaka, et al. 2015). These results have been complemented by reports that AKR1C3 (conversion from A4 to T) and 5α-reductase (reduction of T to DHT) are both expressed in EC (Gibson et al. 2018a; Ito, et al. 2016).

Expression of ERα, ERß1 and splice variant isoforms of ERß (ERß2, ERß5) in EC have been documented (Collins et al. 2019; Collins et al. 2009). In a recent paper we highlighted the potential that ERß5, a variant unable to bind directly to E2, may still influence the response of EC to oestrogens by forming heterodimers with ERα (Collins et al. 2019). High GPER expression is predictive of poor survival in endometrial cancers (Smith, et al. 2007). Prossnitz and colleagues have reported interesting results using ERα-negative/GPER-positive cells which suggest activation of downstream signalling in response to SERMs such as Tamoxifen may explain why women treated with this drug are at higher risk of EC (Petrie, et al. 2013). We, and others, have reported widespread expression of AR in EC (reviewed in (Gibson, et al. 2014)). Evidence that loss of AR is associated with poorer prognosis, reports that AR was elevated in metastases (Kamal, et al. 2016), and that androgens may be anti-proliferative in EC cells have raised the prospect that SARMs should be explored for this cancer as well as those of breast (see below). There are no reports of AR variants being expressed in EC.

Endometriosis

Endometriosis is an oestrogen-dependent neuroinflammatory pain disorder characterised by the presence of 'lesions' of endometrial-like tissue in sites outside the uterus (Horne and Saunders 2019). Endometriosis and adenomyosis are often found in the same patient and may share a common aetiology (Yovich, et al. 2019). Infertility is a common co-morbitity of endometriosis and differences between expression profiles of mRNAs, microRNAs and proteins in endometrial biopsies from controls and women with endometriosis have been reported (Burney, et al. 2009; Burney, et al. 2007) and have recently been reviewed (Bulun, et al. 2019). Notably there remain differing views as to whether receptivity is or is not affected (Lessey and Kim 2017; Miravet-Valenciano, et al. 2017). Studies comparing the impact of a decidualisation stimulus on isolated endometrial stromal cells have reported alterations in the expression of steroidogenic enzymes in cells from women with endometriosis (Aghajanova, et al. 2009). Blunted responses to progesterone,

often termed 'progesterone resistance', are considered a hallmark of the disorder (Aghajanova, et al. 2010; Bulun, et al. 2010). Some have questioned whether this property is an innate feature of the eutopic endometrial cells or acquired when they grow in ectopic sites (McKinnon, et al. 2018).

Some of the best evidence for the importance of intracrine action of steroids has come from studies comparing concentrations of steroids in lesions and eutopic endometrium in women with endometriosis (Huhtinen et al. 2014; Huhtinen et al. 2012). To complement mass spectrometry data, expression of enzymes in lesions such as aromatase, AKR1C3 and STS has been measured with evidence that their over-expression is responsible for generation of a lesion tissue environment rich in oestrogens that can bind ERs or GPER (Rizner 2009, 2016). Notably, aromatase appears to be involved in local biosynthesis of both E2 and the pro-inflammatory regulator prostaglandin E2 (Attar and Bulun 2006). Upregulation of ERß is also considered a hallmark of the altered microenvironment of lesions, which may promote the impact of oestrogens on inflammation, angiogenesis or pain pathways (Bulun, et al. 2012; Greaves, et al. 2014a; Greaves, et al. 2014b).

Adenomyosis

Adenomyosis is a condition characterised by the presence of heterotopic endometrial glands and stroma within the myometrium and has traditionally been difficult to diagnose as it can present with symptoms such as infertility, pain and heavy menstrual bleeding, which are also characteristics of other conditions, including endometriosis and fibroids (Pontis, et al. 2016). Recent advances in imaging offer hope for improved understanding of its presentation and pathogenesis (Chapron, et al. 2020). Altered gene expression in the endometrium of women with adenomyosis has been reported although results have been based on small numbers of samples (Herndon, et al. 2016; Xiang, et al. 2019). It has been suggested that development of adenomyosis may involve mechanisms activated but not resolved during endometrial tissue injury with a common aetiology to some forms of endometriosis (Donnez, et al. 2019; Donnez, et al. 2018). Studies using tissue recovered from women with adenomyosis have identified increased expression of GPER and some association between GPER polymorphisms with the disease, however, it must be noted that study populations have been small (Hong, et al. 2019; Li, et al. 2017). In vitro studies have identified pathways promoting E2-induced overproliferation of uterine smooth muscle cells from women with adenomyosis (Sun, et al. 2015). Immunostaining of tissue sections from adenomyosis uteri have detected changes in ERα, reduced PR and elevated expression of ERβ (Mehasseb, et al. 2011) and aromatase (Barcena de Arellano, et al. 2013) all consistent with an oestrogen-dependent disease. In older papers, expression of AR has been reported (Horie, et al. 1992).

Drugs targeting sex steroid metabolism

Aromatase inhibitors

An excellent historical summary of the discovery of aromatase, identification of increased expression in quadrants of breast containing a tumour, and the development and refinement of aromatase inhibitors (AIs) has been published by leaders in the field (Santen, et al. 2009). The development of highly effective 3rd generation AIs (anastrozole, letrozole, exemestane) led to clinical trials for a number of indications including postmenopausal breast cancer, gynaecomastia in men and ovarian cancer (Langdon, et al. 2017; Miller, et al. 2001; Santen et al. 2009). One key reproducible finding has been a lower rate of EC and venous thrombosis in women treated with AIs compared with those treated with Tamoxifen (Chlebowski, et al. 2015). The ClinicalTrials.gov

website lists 22 trials with search terms endometrial cancer+aromatase inhibitor with the main focus being on women with more advanced disease. Many trials are not yet completed but evidence of benefit in some ER+ cancers has been reported. For example, in 40 women treated with exemestane, there was remission in 10% and lack of progression after 6 months in 35% of the patients (Lindemann, et al. 2014). The PARAGON trial a phase 2 open label study using anastazole in 82 patients with ER and/or PR positive hormonal therapy naive metastatic endometrial cancer reported clinical benefit in 44% (Mileshkin, et al. 2019) although results from other trials have been disappointing and may have been influenced by obesity in the target population (van Weelden, et al. 2019). Some promising results have been reported following treatment of women with the rarer cancer low grade endometrial sarcoma with AIs (reviewed in (Pannier, et al. 2019).

Letrozole and anastrozole have also been evaluated in both pre- and postmenopausal women with endometriosis (Pavone and Bulun 2012). These authors propose that AIs appear to be a suitable therapy for endometriosis-associated pain in women who are postmenopausal by targeting the intracrine oestrogen biosynthesis that contributes to sustained symptoms in this age group. Recent advances have included development of vaginal ring delivery systems for co-administration of anastrozole and the androgenic progestin levonorgestrel (LNG) as a potential therapy for endometriosis associated pain: a phase I trial reported promising findings (Schultze-Mosgau, et al. 2016). Whilst these results seem promising a recent ESHRE guideline that considered whether AIs should be given in combination with contraceptives or other therapies concluded that due to side effects they should only be prescribed to women after all other options for medical or surgical treatment are exhausted (Dunselman, et al. 2014). AIs have also been suggested as therapies for adenomyosis but with the caveat that further studies are required (Vannuccini, et al. 2018).

Sulphatase inhibitors

A number of potent STS inhibitors have been developed with the primary indication being treatment of hormone-dependent cancers (Day, et al. 2009; Purohit and Foster 2012). The compound STX64 (Irosustat**)** was effective in blocking oestrogen synthesis in endometrial cancer cells *in vitro* and was tested as a therapy for advanced endometrial cancer before being discontinued as a mono-therapy by Ipsen (Pautier, et al. 2017). Irosustat has recently been used as an addition to aromatase inhibitors in women with advanced ER+ breast cancer and reported as having a positive clinical impact (Palmieri, et al. 2017). Another inhibitor, estradiol-3-O-sufamate (E2MATE) has been reported which deceased STS activity in human endometrial explants and decreased lesion weight and size but did not alter systemic oestrogens in a mouse model of endometriosis (Colette, et al. 2011). E2MATE, under the trade name PGL2001, has been shown to reduce STS activity in endometrium when given once a week for 4 weeks (Pohl, et al. 2014) the same drug was used in a trial for treatment of endometriosis-associated pain [NCT01631981] but results have not been reported.

Hydroxysteroid dehydrogenase inhibitors

17ßHSD1 inhibitors were originally developed to target the biosynthesis of bioactive E2 in hormone-dependent breast cancer (Day, et al. 2008). Recently, with evidence for expression of 17ßHSD1 in endometriosis lesions, their use has been expanded to treatment of endometriosis with promising results reported (Delvoux, et al. 2014). The role of 17ßHSD5/AKR1C3 in metabolism of both steroids and prostaglandins implicated in endometriosis-associated pain have make it an

attractive target as a novel therapy for this disorder. A number of inhibitors have been developed with the Bayer compound BAY1128688 showing sufficient promise for it to be used in a phase 2 randomised clinical trial to assess efficacy of different doses in 121 women with symptomatic endometriosis. The trial [NCT03373422] was terminated after 8 months due to an increased incidence of liver toxicity highlighting the challenge of developing drugs that may target enzymes present in multiple tissues (van Weelden et al. 2019). In their recent review Rizner and Penning (Rizner and Penning 2020) concluded the 'hepatotoxicity the effect was probably compound related which does not preclude AKR1C3 as a target' and development of other drugs targeting this enzyme alone or in combination with other targets is continuing (Wangtrakuldee, et al. 2019)

Dual/combined targeting

Whilst initial studies have focused on mono-therapies, a new generation of drugs with dual actions has also been developed – examples include those that target aromatase and STS (DASI, (Purohit and Foster 2012)) or STS and 17βHSD1. Whilst some in vitro studies seem promising, clinical trials are yet to be completed (reviewed in (Potter 2018)).

Drugs targeting oestrogen and androgen receptors and their potential to treat endometrial disorders

The solving of the crystal structure of nuclear ERs as well as detailed modelling of the impact of ligand binding on conformation, recruitment of co-factors and gene expression laid the foundation for the development of synthetic ligands that exhibit selectivity, tissue-specific agonism, antagonism or induce receptor degradation; a comprehensive perspective and background is provided by Burris et al 2013 (Burris, et al. 2013). Table 2 summarises the specificities and properties of some of the novel non-steroidal ligands developed to target ERs and AR a number of which have been investigated in the context of endometrial disorders and are discussed below.

Oestrogen receptors

Agonists and antagonists with selectivity for ERα, ERß and GPER have been validated using a range of cell based and animal models (Table 2). When Frasor et al (Frasor, et al. 2003) compared the effect of 4x daily injections of 4,4',4''-(4-Propyl-[1*H*]-pyrazole-1,3,5-triyl)*tris*phenol (PPT) or 2,3-*bis*(4-Hydroxyphenyl)-propionitrile (DPN) to immature (d21) female mice, they noted differences in tissue response which they attributed to activation of ERα or ERß respectively. PPT caused epithelial cell proliferation, increased uterine weight and expression of lactoferrin but decreased *Ar* mRNA. In contrast, DPN did not increase uterine weight or luminal epithelial cell proliferation but appeared able to reduce stimulation by PPT. These findings are consistent with a large body of work that implicates ERα as the major regulator of oestrogen-dependent proliferation in the uterus (Hewitt and Korach 2003; Winuthayanon, et al. 2017). In contrast, it appears that ERß may have other functions including specific roles in inflammation and angiogenesis (Critchley et al. 2001; Gibson et al. 2015; Gibson and Saunders 2012; Greaves et al. 2013). There have been fewer studies focussed on GPER but when Zhang et al (Zhang et al. 2017) treated primary endometrial stromal cells with E2, G1 ((±)-1-[(3a*R**,4*S**,9b*S**)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetrahydro-3*H*-cyclopenta[*c*]quinolin-8-yl]- ethanone) or G15 ((3a*S**,4*R**,9b*R**)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-3*H*-cyclopenta[*c*]quinolone), they reported that stimulation of GPER with G1 mimicked the impact of E2 and resulting in stabilisation of HIF protein and increased

457 expression of VEGF and MMP9. The Prossnitz group generated a GPER antagonist (G36, (±)-
458 (3a*R**,4*S**,9b*S**)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetrahydro-8-(1-methylethyl)-3*H*-
459 cyclopenta[*c*]quinolone, Table 2) with improved selectivity and reported that it can block multiple
460 E2-mediated second messenger signalling pathways and endometrial cell proliferation (Dennis, et
461 al. 2011).

462 Selective oestrogen receptor modulators (SERMs) were developed to treat ERα-positive breast
463 cancers with the ideal SERM being one that acts as an antagonist in breast but an agonist in bone
464 (Burris et al. 2013). The evolution in our understanding of tissue selective activities of ligand-
465 activated receptors coupled with the discovery of different ER subtypes and splice variants has
466 resulted in several generations of SERMs. Tamoxifen is a first generation SERM that displays
467 agonism in the endometrium, increasing EC risk; second generation SERMs such as Raloxifene do
468 not agonize endometrial growth and are associated with lower risk of EC and may have additional
469 positive effects on cognition and the cardiovascular system (Muchmore 2000). Other SERMs have
470 a mixture of agonist/antagonist activity in endometrium, agonist activity in bone and antagonism in
471 breast (Pickar, et al. 2018).

472 Selective oestrogen receptor degraders (SERDs) antagonize ERα and induce its degradation,
473 resulting in a decrease in ERα protein levels: they do not show agonist properties in other tissues
474 (Kieser, et al. 2010). Fulvestrant was the first SERD to be approved as a therapeutic and is
475 commonly used as a treatment for advanced breast cancer (Blackburn, et al. 2018). Although
476 originally marketed under the trade name Faslodex by AstraZeneca manufacture of generic versions
477 has been approved by the US Federal Drugs Administration. A number of new generation SERDs
478 are in development (Pepermans and Prossnitz 2019) one of which is bazedoxifene (BZA) a
479 compound which exhibits SERD properties in breast cancer with beneficial properties in bone and
480 no adverse impact on endometrium leading to its approval for hormone replacement therapies
481 (Pickar et al. 2018). Recent mechanistic studies suggest BZA may be useful in treating cancers
482 which contain ERα mutants (Fanning, et al. 2018). In addition to activation by endogenous
483 oestrogens there is evidence that GPER may also be activated by SERMs/SERDs developed to
484 target ERα which may explain some apparent discordant results in ERα negative cancers (see
485 review by (Meyer, et al. 2011).

486 *Androgen receptors*

487 Selective androgen receptor modulators (SARMs) have been developed to support the beneficial
488 impacts of AR-mediated cell function in bone and muscle without the adverse side effects seen with
489 high doses T or DHT (gynaecomastia, aggression, prostate hyperplasia) (Burris et al. 2013;
490 McEwan 2013) (Table 2). New generation SARMs have been proposed as therapeutics for women
491 suffering from breast cancer, muscle wasting or urinary incontinence and a number of clinical trials
492 have been undertaken to evaluate their use for these indications (Brodie and McEwan 2005; Dalton,
493 et al. 2011).

494 *Targeting oestrogen receptors in endometrial disorders*

495 A high proportion of low grade EC express ERα as well as progesterone receptors. In a recent
496 systematic review van Weelden and colleagues highlighted the progestins as a first line hormonal
497 therapy and use of antioestrogens as an alternative therapy option highlighting results from 10 trials

using SERMs or SERDs as monotherapies between 1981 and 2013 (van Weelden et al. 2019). Studies all showed some beneficial response to therapy although results were variable and the authors concluded that Tamoxifen or a combination of tamoxifen and progestin might be the best choice when selecting second line hormonal treatment. In subsequent studies the SERM Ospemifene has been shown as an effective in treatment of vaginal symptoms in postmenopausal women (Archer, et al. 2019) and only acts as an agonist in endometrium in high doses. The SERD fulvestrant/faslodex (Table 2) has been investigated as a treatment for endometrial cancer in phase I/II trials, although well tolerated, it has low oral bioavailability and further trials are needed (Bogliolo, et al. 2017). Another recent study suggested dual targeting of ERα with tamoxifen and ERRα with XCT790 may be beneficial for EC treatment but this requires further validation (Mao, et al. 2019).

Whilst administration of SERMs/SERDs may be appropriate for postmenopausal women with cancer, their use in younger women with non-malignant endometrial disorders such as endometriosis is more challenging with data limited to promising results in preclinical models (Khine, et al. 2018; Kulak, et al. 2011). The observation that ERß is highly expressed in endometriosis lesions and the development of ERß-selective agonists such as ERß-041 with apparent anti-inflammatory properties provided a rationale for testing them as therapies for endometriosis with promising results obtained in pre-clinical models (Harris 2006). Several clinical trials were conducted with ERß-041 but no positive outcomes were reported. In a recent review Guo and Groothuis highlighted a number of reasons why drugs targeting ERß including the SERM Fulvestrant and ERß-041 failed to deliver the patient benefit in clinical trials. The reasons highlighted included, but were not limited to, animal models that did not recapitulate long-established disease, translation of dose from rodent to women and incomplete understanding of the role of ERß antagonism in pain mechanisms (Guo and Groothuis 2018). SERMs are not considered suitable therapies for adenomyosis (Pontis et al. 2016). The SERM Ormeloxifene developed for use as a contraceptive, has also shown promising results in treating heavy menstrual bleeding (HMB) in perimenopausal women in India (Pati, et al. 2017).

GPER has also been investigated as a target for treatment of endometriosis with reports that the GPER agonist G-1 induced cell cycle arrest and apoptosis of stromal cells derived from ovarian endometriosis cysts (Mori, et al. 2015). GPER has been implicated in E2-stimulated nociceptive pain in endometriosis with results in a mouse model showing administration of the selective GPER antagonist G36 inhibited the pain response (Alvarez, et al. 2014). Properly designed clinical trials are needed to explore GPER as a target for relief of painful symptoms in endometriosis in women.

Targeting androgen receptors in endometrial disorders

The development of SARMs has prompted renewed interest in targeting of AR in reproductive disorders whilst also raising concerns related to side effects including hirsutism that are a hallmark of excess androgens in PCOS. Transgender individuals may be one group who might benefit from SARMs as administration of high concentrations of testosterone can result in abnormal uterine bleeding and metabolism to oestrogen may explain increased rates of endometrial cancer (Grimstad, et al. 2019) but there are no registered clinical trials.

Danazol is a synthetic androgen first used as a treatment in the 1970s: it binds AR with high affinity and is also reported to reduce the activity of a number of enzymes including steroid sulphatase

(Carlstrom, et al. 1984). Danazol has anti-proliferative effects on uterine cells (Kauppila, et al. 1985). A systematic review of RCTs using Danazol to treat endometriosis concluded that treatment was associated with reduced lesion size and relief of pain symptoms and women who took Danazol were more satisfied with their treatment compared with women who had placebo treatment (Selak, et al. 2007). The antiproliferative and hormone-suppressive activities of Danazol has formed the basis of treatments for adenomyosis (Vannuccini et al. 2018) and heavy menstrual bleeding (Beaumont, et al. 2007) with efficacy being demonstrated. The androgenic activity of Danazol is associated with side effects including hirsutism and deepening of the voice and it is contraindicated for women at risk of pregnancy because of the risk of virilisation of the fetus (Selak et al. 2007). These side effects have limited its use and prompted efforts to develop therapies that are less virilising.

Using a mouse model we have compared the impact of DHT with Danazol and new generation SARMs GTx-024 and GTx-007 (Table 2) and found that both Danazol and GTx-024 restored uterine weight of ovariectomised female mice to that of intact animals, while GTx-007 had no similar effect (Simitsidellis, et al. 2019). These preclinical studies highlight the importance of considering impacts on the endometrium when women are included in clinical trials using SARMs (Dalton et al. 2011; Neil, et al. 2018). Whilst SARMs have been used in clinical trials for treatment of breast cancer they have not as yet been tested as treatments for endometrial cancer or endometriosis (Narayanan, et al. 2018). Standard medical treatment for HMB involves targeting of the progesterone receptor either with the androgenic progestagen levonorgestrol delivered in an intra-uterine device or with newly developed selective progesterone receptor modulators (SPRMs; (Maybin and Critchley 2016)). Interestingly, administration of progesterone receptor antagonists or SPRMs such as UPA (ulipristal acetate) as a treatment for heavy menstrual bleeding results in a significant increase in expression of AR (Whitaker, et al. 2017) which may in part explain their antiproliferative action. Treatment with new generation SARMs is yet to be investigated.

Summary and Future Directions

The endometrium is a dynamic tissue which, by virtue of its expression of high affinity receptors, is exquisitely sensitive to the actions of oestrogens and androgens. Temporal and spatial changes in tissue function in response to steroids play a critical role in preparation for pregnancy and in breakdown and shedding if pregnancy does not occur. Balanced regulation of sex steroid action is essential for endometrial function and is controlled via local metabolism and cell- and tissue-specific expression of steroid receptors/isoforms. Drugs targeting steroid metabolising enzyme activity and/or receptor function have reported efficacy in several endometrial disorders, but their use has often been limited due to lack of tissue specificity and undesirable side effect profiles. Recent development of drugs that selectively target steroid receptors such as next generation SERMs, SERDs, SARMs and SARDs show promise as new therapeutics but further pre-clinical studies and clinical trials are needed to determine if these drugs have efficacy specifically for the indication of endometrial disorders.

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582 **Declaration of interest**

583 We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality
584 of the research reported

585

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Table Legends

Table 1. Hormone-dependent endometrial pathologies in women.
For each pathology an estimate of incidence, hallmark features and one or two key references are summarised.

Table 2. Non-steroidal drugs targeting oestrogen and androgen receptors.
Each drug is identified by its common abbreviation or registered name, the activity as reported in the literature, whether it has been used in one or more clinical trial(s) and a key reference is provided. Abbreviations: PPT - 4,4',4''-(4-Propyl-[1*H*]-pyrazole-1,3,5-triyl)*tris*phenol; DPN - 2,3-*bis*(4-Hydroxyphenyl)-propionitrile; GPER - G protein-coupled estrogen receptor 1; G36 - (±)-(3a*R**,4*S**,9b*S**)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetrahydro-8-(1-methylethyl)-3*H*-cyclopenta[*c*]quinoline; SERM – selective oestrogen receptor modulator; SERD – selective oestrogen receptor degrader; SARM – selective androgen receptor modulator

Figure Legends.

Figure 1. Expression of oestrogen receptor alpha (ERα) and androgen receptor (AR) in full thickness samples from the human uterus.

The tissue is divided into functional and basal layers supported on the myometrium below and bounded on upper surface by the luminal epithelium. ERα (red stain) is abundant in epithelial cells in the proliferative phase but down-regulated in the secretory phase. AR (green) is localised to stromal cells in the basal and functional layers during the proliferative phase but only expressed in the basal stromal cells in the secretory phase when its expression is upregulated in epithelial cells. P – proliferative phase, S – secretory phase, M – menstrual phase. Adapted from Marshall et al 2011.

Figure 2. Simplified diagram of key biosynthetic steroids implicated in intracrine biosynthesis of oestrogens and androgens within endometrial tissue.

In pre-menopausal women both the adrenal and ovary are the primary sites of biosynthesis of steroids. Expression of all enzymes illustrated has been validated in human tissue or primary endometrial stromal cells exposed to a decidualisation stimulus (Gibson et al. 2013; Gibson et al. 2016a; Gibson et al. 2018b). For a more comprehensive steroidogenic pathway readers are referred to the review by (Konings et al. 2018).

Endometrial pathology	**Incidence**	**Features**	**References**
Implantation failure, recurrent pregnancy loss	1 in 6 couples are infertile rates of implantation failure difficult to determine other than in IVF, RPL 1-2%	Poor/out of phase decidual response. Changes in immune cell cohorts (uNK?). Stromal cell senescence with age?	(Quenby et al. 2009); (Lucas, et al. 2020)
Heavy menstrual bleeding (HMB)	20-30% of women; may be worse during perimenopause; associated with fibroids	Acute or chronic; FIGO classification of causes (Palm-Coen)	(Whitaker and Critchley 2016)
Endometriosis	~10% women of reproductive age; may be asymptomatic. 40% of infertile patients may have endometriosis	3 subtypes – aetiology may be different. Neuroinflammation and chronic pain. Changes in peritoneal environment.	(Horne and Saunders 2019, 2020; Horne, et al. 2017)
Adenomyosis	~20% in women in gynaecology clinics (higher in older women)	Growth of endometrial fragments within myometrial wall. Myometrial thickening on ultrasound. Association with endometriosis.	(Naftalin, et al. 2012)
Asherman's Disease	Estimates of incidence vary widely: 3 to 45 % in infertile population?	Adhesions within uterine cavity; risk increased by endometrial ablation/surgery	(Dreisler and Kjer 2019).
Endometrial hyperplasia		Increase in gland to stroma ratio when compared with proliferative endometrium. Some types may progress to endoCa	(Sanderson et al. 2017)
Endometrial cancer	4th most common cancer in women UK; rates rising	Risk increased by high BMI and Lynch syndrome. Classifications based on histology or genetics with 'unopposed' oestrogen key risk factor for some subtypes.	(Sanderson et al. 2017); (Ryan, et al. 2017)

Name	Receptor activity	Clinical Trials?	References
PPT	ERα selective agonist	Stimulates epithelial cell proliferation.	(Frasor, et al. 2004)
DPN	ERbeta selective agonist	Stimulates endometrial endothelial cells	(Greaves et al. 2013)
LNS8801	GPER agonist	Phase 1 open label clinical trial in advanced solid and hematologic cancers.	NCT04130516
G36	GPER antagonist	Improved selectivity compared to G15.	(Dennis et al. 2011)
Tamoxifen	SERM	Treatment and prevention of ERα-positive breast cancers in pre- and post-menopausal women. Agonist action in endometrium	(Jordan 2003)
Raloxifene, Evista	SERM	Prevention of invasive breast cancer in post-menopausal women. Positive effects on bone, cognition, cardiovascular system.	(Muchmore 2000)
Fulvestrant, Faslodex	SERD	Licensed as first line endocrine management for advanced breast cancer in post-menopausal women	(Blackburn, et al. 2018)
Bazedoxifene, Duavee	SERM/SERD	Positive impacts on bone, approved for HRT, SERD in endometrium	(Fanning et al. 2018)
GTx24, Enobosarm,	SARM	Muscle wasting in cancer, breast cancer,	(Gao and Dalton 2007)

		urinary stress incontinence	
GTx007, Andarine,	SARM, partial agonist	Tested in preclinical models; issues with use in doping	(Kearbey, et al. 2007)
GSK2881078	SARM, long half life	Muscle loss in patients with chronic disease (discontinued) Improved muscle mass in healthy women	(Neil et al. 2018)
AZD3514	SARD	Moderate anti-tumour activity in advanced castrate-resistant PCa. Significant levels of nausea and vomiting.	(Omlin, et al. 2015)

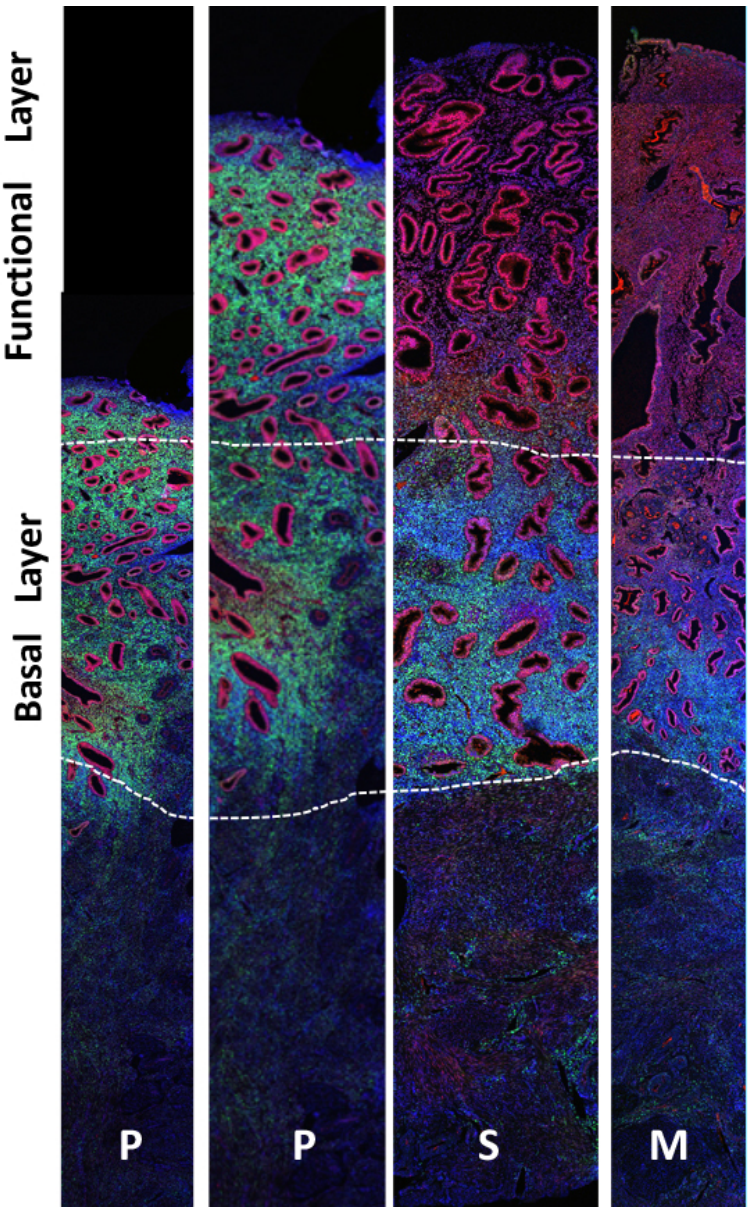

Figure 1. Expression of oestrogen receptor alpha (ERα) and androgen receptor (AR) in full thickness samples from the human uterus.
The tissue is divided into functional and basal layers supported on the myometrium below and bounded on upper surface by the luminal epithelium. ERα (red stain) is abundant in epithelial cells in the proliferative phase but down-regulated in the secretory phase. AR (green) is localised to stromal cells in the basal and functional layers during the proliferative phase but only expressed in the basal stromal cells in the secretory phase when its expression is upregulated in epithelial cells. P – proliferative phase, S – secretory phase, M – menstrual phase. Adapted from Marshall et al 2011.

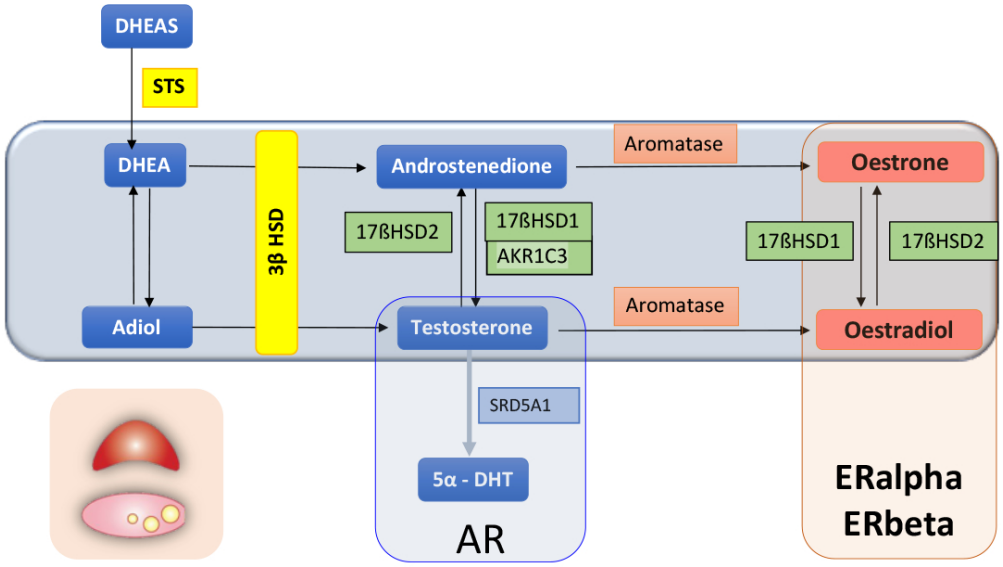

Figure 2. Simplified diagram of key biosynthetic steroids implicated in intracrine biosynthesis of oestrogens and androgens within endometrial tissue.
In pre-menopausal women both the adrenal and ovary are the primary sites of biosynthesis of steroids. Expression of all enzymes illustrated has been validated in human tissue or primary endometrial stromal cells exposed to a decidualisation stimulus (Gibson et al. 2013; Gibson et al. 2016a; Gibson et al. 2018b). For a more comprehensive steroidogenic pathway readers are referred to the review by (Konings et al. 2018).